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Association of Herpes Group of Viruses with Oral Cancer

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Introduction: Association of Herpes Simplex Virus type 2 (HSV-2) with human uterine cervical cancer has been firmly established (1,2). Oral cavity, as in the case of uterine carvix is a major site of recurrent herpetic infection. Oral cancer constitutes about 27% of the total cancer cases registered in this region (3). This is one of the highest prevalence rates seen anywhere in the world. Oral cancer has been shown to be associated with chewing tobacco, but other factors, including viruses as etiological agents, are to be carefully evaluated. We had elsewhere reported that the antibodies against HSV-1 are increased in oral cancer patients (4,5) and the oral cancer cells contain HSV-1 related antigens (6) and HSV genes are present in the oral cancer cells (7). Human herpes virus-6 (HHV-6), was first isolated from the peripheral blood lymphocytes of patients with lymphoproliferative disorders and AIDS (8). Elevated levels of anti HHV-6 antibodies have been found in patients with Exanthum subitum, Kikuchi's mononucleosis, AIDS and in lymphoid malignancies (9).

Materials and Methods: Presence of antibody against HSV-1 was determined by indirect Hemagglutination micro neutralisation or ELISA tests.

The presence of IgG antibodies to the viral capsid antigen of the Z29 strain of HHV-6 and the antibody titre in serum were evaluated using immunoperoxidase technique. Molt-3 cells, infected with HHV-6 in culture, were used as the source of HHV-6 viral capsid antigen (10). The Molt-3 cells were harvested, washed, smeared on teflon-coated slides, fixed in cold acetone for 10 min and stored, and Immunoperoxidase staining was carried out, using patient's serum at different dilutions. Endogenoeus peroxidase activity was blocked by treating the smears with H202.

Single cell suspensions were prepared from punch biopsy specimens from oral cancers. These cells were washed, smears were prepared, air dried and fixed in acetone for 10 minutes. Indirect immunofluorescence was done using specific HSV-1 antiserum.

ECoRI fragments, HSV-1 D, HSV-1 I and HSV-1 M-A were cloned in PGEM 32 plasmid vector and the ECoRI fragment (E and K) was cloned

in PBR 325 plasmid vector. The cloned HSV-1 fragments were nick translated by employing P32-dCTP and used for dot-blot hybridisation. The HSV-1 fragments D and I were used for in situ hybridisation. Random priming method was employed for probe synthesis, using S35-dCTP.

Results and Discussion: Prevalence of anti-HSV-1 antibodies in different groups are shown in Table 1. Out of 919 oral cancer patients 652 (71%) showed the presence of anti-HSV-1 antibodies, as against 52% in healthy control subjects ($P > 0.001$). Both HSV-1 and adenovirus infections are common in this region, as shown by the control values. But only HSV-1 antibodies, but not the adenovirus antibodies, did show any increase in oral cancer group. The anti-HSV-1 antibodies were not only more prevalent in cancer group, but the patients had increased titre values also.

The results of studies on seroprevalence of HHV-6 are shown in Table II. The prevalence of anti-HHV-6 antibodies in normal control was 78%, and the titre ranged from 10 to 160 (mean, 48). In HL and ALL, the antibodies could be detected in all the serum samples, with litres of 320 to 5120 (mean, 1109) and 160 to 5120 (mean, 1208) respectively. In oral cancer, the prevalence was elevated to 96% and the litre was very high, 80 to 10250 (mean, 2042).

The results of immunofluorescence showed that out of 20 normal control specimens, only 30% showed fluorescence; but 135/175 (=77%) oral cancer specimens showed brilliant fluorescence. The immunoperoxidase staining also confirmed these results.

The results of DNA hybridisation are given in Table III. HSV probes were hybridised with 64% oral cancer cases and 38% cervical cancer cases. Further, Human papilloma virus (HPV-16) was seen to be hybridised with (8%) oral cancer and 36% of cervical cancer specimens. The normal cell DNA did not hybridise with viral probes.

The above experiments strongly show the relationship of Herpes viruses with oral cancer. The HSV-1 antibodies are consistently higher in oral cancer patients, the HSV-1 related antigens are shown to be on the oral cancer cells and the HSV-1 probe could be hybridised with the DNA from oral cancer specimens. HHV-6 antibodies are also high in oral cancer patients.

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TABLE-1: SERUM ANTIBODIES AGAINST HERPES SIMPLEX VIRUS TYPE-1 IN CANCER PATIENTS

Groups studied	Total No. of sera	No. of sera Positive	% +ve	P. Value
Oral Cancer	919	652	71	>0.001
Cervical Cancer	586	228	56	-
Other Cancers	890	432	49	-
Normal controls	650	338	52	-

TABLE II: SEROPREVALENCE AND TITRE OF ANTI-HHV-6 ANTIBODY

Groups	No. of samples studied	Prevalence	Antibody titre	
			Range	Mean
Normal	150	78%	10-160	48
NHL	59	95%	10-320	88
HL	28	100%	320-5120	1109
ALL	51	100%	160-5120	1208
Cervix Cancer	50	74%	10-160	53
Breast Cancer	50	78%	10-160	62
Oral Cancer	127	96%	80-10240	2042

NHL = Non-Hodgkin's Lymphoma; HL = Hodgkin's Lymphoma;
ALL = Acute Lymphoblastic Leukemia

TABLE-III: RESULTS OF DOT-BLOT AND IN-SITU DNA HYBRIDISATION STUDIES

Technique	Probe	Normal controls positive	Oral cancer specimens positive	Cervical Cancer Specimens positive
Dot blot	HSV-1-M-A	5/25/(20%)	51/80(64%)	-
Dot blot	HSV-1-E+K	2/25(8%)	45/80(56%)	-
Dot blot	HSV-1-D	2/25(8%)	34/60(57%)	-
In situ	HSV-1-D	3/25(12%)	48/80(60%)	-
In situ	HSV-1-1	3/25(12%)	48/80(60%)	-
Dot blot	HSV-2-Bgl-II-N	2/25(8%)	-	19/50 (38%)
Dot blot	HPV-16	2/25(8%)	-	18/50 (36%)